

Claims

We claim:

1. An isolated protein selected from the group consisting of:

a) proteins comprising the sequence of amino acids of SEQ ID NO: 2 from amino acid residue 45 to amino acid residue 196;

b) proteins comprising the sequence of amino acids of SEQ ID NO:2 from amino acid residue 45 to amino acid residue 206;

c) proteins comprising the sequence of amino acids of SEQ ID NO: 19 from amino acid residue 22 to amino acid residue 173;

d) proteins comprising the sequence of amino acids of SEQ ID NO:19 from amino acid residue 22 to amino acid residue 175;

e) allelic variants of (a), (b), (c) or (d); and

f) species homologs of (a), (b), (c), (d) or (e) wherein said protein stimulates proliferation or differentiation of myeloid or lymphoid precursors.

2. An isolated protein according to claim 1, wherein said protein comprises the sequence of amino acids of SEQ ID NO:2 from amino acid residue 45 to amino acid residue 379.

3. An isolated protein according to claim 1, wherein said protein comprises the sequence of amino acids of SEQ ID NO:19 from amino acid residue 22 to amino acid residue 353.

4. An isolated protein according to claim 1 wherein said protein is a mouse protein.

5. An isolated protein according to claim 1 wherein said protein is a human protein.

6. An isolated protein according to claim 1, wherein said protein comprises:

the sequence of amino acids shown in SEQ ID NO: 2 from amino acid residue 45 to residue 379;

the sequence of amino acids shown in SEQ ID NO: 2 from amino acid residue 24 to residue 196;

the sequence of amino acids shown in SEQ ID NO: 2 from amino acid residue 24 to residue 206;

the sequence of amino acids shown in SEQ ID NO: 2 from amino acid residue 24 to residue 379;

the sequence of amino acids shown in SEQ ID NO: 2 from amino acid residue 1 to residue 196;

the sequence of amino acids shown in SEQ ID NO: 2 from amino acid residue 1 to residue 206; or

the sequence of amino acids shown in SEQ ID NO: 2 from amino acid residue 1 to residue 379.

7. An isolated protein according to claim 1, wherein said protein comprises:

the sequence of amino acids shown in SEQ ID NO: 19 from amino acid residue 1 to residue 173;

the sequence of amino acids shown in SEQ ID NO: 19 from amino acid residue 1 to residue 175;

the sequence of amino acids shown in SEQ ID NO: 19 from amino acid residue 1 to residue 353; or

the sequence of amino acids shown in SEQ ID NO: 19 from amino acid residue 22 to residue 353.

8. An isolated protein consisting essentially of a sequence of amino acids selected from the group consisting of:

the sequence of amino acids shown in SEQ ID NO: 2 from amino acid residue 45 to amino acid residue 196;

the sequence of amino acids of SEQ ID NO:2 from amino acid residue 45 to amino acid residue 206;

the sequence of amino acids of SEQ ID NO:2 from amino acid residue 45 to amino acid residue 379;

the sequence of amino acids of SEQ ID NO:19 from amino acid residue 22 to amino acid residue 175; and

the sequence of amino acids of SEQ ID NO:19 from amino acid residue 22 to amino acid residue 353.

9. An isolated protein that stimulates the proliferation or differentiation of myeloid or lymphoid precursors, wherein said protein comprises a segment that is at least 80% identical at the amino acid level to the sequence of amino acids of SEQ ID NO:2 from amino acid residue 45 to amino acid residue 196 or the sequence of amino acids of SEQ ID NO:19 from amino acid residue 22 to amino acid residue 173.

10. An isolated polynucleotide molecule encoding a protein according to claim 1.

*sub a'*  
11. An isolated polynucleotide molecule according to claim 10 wherein said molecule is a DNA molecule comprising a coding strand comprising the sequence of nucleotides of SEQ ID NO:1 from nucleotide 237 to nucleotide 692.

*a*  
12. An isolated polynucleotide molecule according to claim 10 wherein said molecule is a DNA molecule comprising a coding strand comprising the sequence of nucleotides of SEQ ID NO:18 from nucleotide 64 to nucleotide 519.

*sub a2*  
13. An isolated polynucleotide molecule according to claim 10 wherein said molecule encodes the amino acid sequence of SEQ ID NO:2 from amino acid residue 45 to amino acid residue 196.

14. An isolated polynucleotide molecule according to claim 10 wherein said molecule encodes the amino acid sequence of SEQ ID NO:19 from amino acid residue 22 to amino acid residue 173.

15. An isolated polynucleotide molecule selected from the group consisting of:

(a) DNA molecules encoding a hematopoietic protein and comprising a nucleotide sequence as shown in SEQ ID NO:1 from nucleotide 237 to nucleotide 692;

(b) DNA molecules encoding a hematopoietic protein and comprising a nucleotide sequence as shown in SEQ ID NO:18 from nucleotide 64 to nucleotide 519;

(c) allelic variants of (a) or (b);

(d) DNA molecules encoding a hematopoietic protein that is at least 80% identical in amino acid sequence to a protein encoded by (a), (b) or (c); and

(e) molecules complementary to (a), (b), (c) or (d).

16. An isolated polynucleotide molecule according to claim 15 wherein said molecule encodes a hematopoietic protein that is at least 90% identical in amino acid sequence to a protein encoded by (a), (b) or (c).

17. An isolated polynucleotide molecule according to claim 15 wherein said molecule comprises nucleotide 237 to nucleotide 722 of SEQ ID NO: 1 or nucleotide 64 to nucleotide 525 of SEQ ID NO: 18.

18. An isolated DNA molecule selected from the group consisting of:

(a) the *Eco* RI-*Xho* I insert of plasmid pZGmpl-1081 (ATCC 69566);

(b) allelic variants of (a); and

(c) DNA molecules encoding a protein that is at least 80% identical in amino acid sequence to a protein

encoded by (a) or (b), wherein said isolated DNA molecule encodes a protein having hematopoietic activity.

19. An isolated DNA molecule according to claim 18 wherein said molecule encodes a polypeptide comprising the amino acid sequence of SEQ ID NO:2 from amino acid residue 45 to amino acid residue 196.

20. An expression vector comprising the following operably linked elements:

a transcription promoter;

a DNA segment selected from the group consisting of:

(a) DNA segments encoding a hematopoietic protein and comprising a nucleotide sequence as shown in SEQ ID NO:1 from nucleotide 237 to nucleotide 692;

(b) DNA segments encoding a hematopoietic protein and comprising a nucleotide sequence as shown in SEQ ID NO:18 from nucleotide 64 to nucleotide 519;

(c) allelic variants of (a) or (b); and

(d) DNA segments encoding a hematopoietic protein that is at least 80% identical in amino acid sequence to a protein encoded by (a), (b) or (c); and

a transcription terminator.

21. An expression vector according to claim 20 wherein said DNA segment encodes a hematopoietic protein that is at least 90% identical in amino acid sequence to a protein encoded by (a), (b) or (c).

22. An expression vector according to claim 20 wherein said DNA segment comprises nucleotide 237 to nucleotide 722 of SEQ ID NO: 1 or nucleotide 64 to nucleotide 525 of SEQ ID NO: 18.

23. An expression vector according to claim 20 further comprising a secretory signal sequence operably linked to the DNA segment.

24. A cultured cell into which has been introduced an expression vector according to claim 20, wherein said cell expresses a hematopoietic protein encoded by <sup>part</sup>the DNA segment.

25. A cultured cell according to claim 24 wherein said cell is a fungal cell.

26. A cultured cell according to claim 25 wherein said cell is a yeast cell.

27. A cultured cell according to claim 24 wherein said cell is a mammalian cell.

28. A cultured cell according to claim 24 wherein said cell is a bacterial cell.

29. A non-human mammal into the germ line of which has been introduced a heterologous DNA segment selected from the group consisting of:

(a) DNA segments encoding a hematopoietic protein and comprising a nucleotide sequence as shown in SEQ ID NO:1 from nucleotide 237 to nucleotide 692;

(b) DNA segments encoding a hematopoietic protein and comprising a nucleotide sequence as shown in SEQ ID NO:18 from nucleotide 64 to nucleotide 519;

(c) allelic variants of (a) or (b); and

(d) DNA segments encoding a hematopoietic protein that is at least 80% identical in amino acid sequence to a protein encoded by (a), (b) or (c);

wherein said mammal produces the hematopoietic protein encoded by said DNA segment.

30. A non-human mammal according to claim 29 selected from the group consisting of pigs, goats, sheep, cattle and mice.

31. A non-human mammal according to claim 29 wherein said DNA segment comprises nucleotide 237 to nucleotide 722 of SEQ ID NO: 1 or nucleotide 64 to nucleotide 525 of SEQ ID NO: 18.

32. A method of producing a hematopoietic protein comprising:

culturing a cell into which has been introduced an expression vector according to claim 20, whereby said cell expresses a hematopoietic protein encoded by the DNA segment; and

recovering the hematopoietic protein.

33. A method according to claim 32 wherein said hematopoietic protein is secreted by said cell and is recovered from a medium in which said cell is cultured.

34. A pharmaceutical composition comprising a protein according to claim 1 in combination with a pharmaceutically acceptable vehicle.

35. An antibody that binds to an epitope of a protein according to claim 1.

36. A method for stimulating platelet production in a mammal comprising administering to said mammal a therapeutically effective amount of a hematopoietic protein selected from the group consisting of:

a) proteins comprising the sequence of amino acids of SEQ ID NO: 2 from amino acid residue 45 to amino acid residue 196;

(b) proteins comprising the sequence of amino acids of SEQ ID NO:19 from amino acid residue 22 to amino acid residue 173;

(c) allelic variants of (a) or (b); and

(d) species homologs of (a), (b) or (c),

wherein said protein stimulates proliferation or differentiation of myeloid or lymphoid precursors, in combination with a pharmaceutically acceptable vehicle.

37. A probe which comprises an oligonucleotide of at least 14 nucleotides, wherein the sequence of said oligonucleotide is at least 80% identical to a same-length portion of:

(a) SEQ ID NO: 1;

(b) SEQ ID NO: 18;

(c) SEQ ID NO: 28; or

(d) sequences complementary to SEQ ID NO: 1, SEQ ID NO: 18 or SEQ ID NO: 28.

38. A method for detecting, in a mixture of DNA molecules, a DNA molecule encoding thrombopoietin comprising probing a mixture of DNA molecules with a probe which comprises an oligonucleotide of at least 14 nucleotides, wherein the sequence of said oligonucleotide is at least 80% identical to a same-length portion of:

(a) SEQ ID NO: 1;

(b) SEQ ID NO: 18;

(c) SEQ ID NO: 28; or

(d) sequences complementary to SEQ ID NO: 1, SEQ ID NO: 18 or SEQ ID NO: 28; and

detecting DNA molecules to which said probe hybridizes.

39. A method for stimulating cell proliferation comprising adding to cultured bone marrow cells an isolated



protein according to claim 1 in an amount sufficient to stimulate cell proliferation.

40. A method according to claim 39 wherein said cells are megakaryocytes or megakaryocyte precursors.

41. A method for purifying thrombopoietin comprising:

exposing a solution containing thrombopoietin to an antibody attached to a solid support, wherein said antibody binds to an epitope of a protein according to claim 1;

washing said antibody to remove unbound contaminants;

eluting bound thrombopoietin from said antibody; and recovering said eluted thrombopoietin.